Rapid Techniques for Allergen Detection

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Agenda

Food Allergens: What and How?
So What?
Tools for Protection?
What Are Food Allergies?

• Naturally occurring proteins
• Heat and processing resistant
• Resistant to extremes in pH
• Usually major proteins in food
• Foods can have 1 or many allergens
• No known cure…strict avoidance
Food Allergy vs. Food Intolerance

Food Allergy: IgE mediated immune response, triggers release of histamine, i.e. watering eyes, running nose, scratchy throat

Food Intolerance: Sensitivity to certain foods caused by a deficiency i.e. lactose intolerance
How Food Allergens Work

• Specific proteins stimulate immune response in two stages
  
A. Initial sensitization (no response)
B. Subsequent exposure (release of histamine)
How Food Allergens Work

1. B cell making IgE to antigen
2. Helper function
3. T cell with T cell receptor to antigen
4. IgE receptor
5. Mast cell binding fragment
6. IgE sensitizes tissue mast cells by binding to their surfaces at IgE receptor site
7. Subsequent exposures to antigen
8. Antigen cross-links two antibody molecules
9. Release of allergic mediators (histamines, serotonin, and so on)
10. Allergies
How Food Allergens Work
Leading Cause of Food Recalls

Food Allergen Recalls

- 2000: 400
- 2001: 150
- 2002: 300
- 2003: 250
- 2004: 200
- 2005: 150
- 2006: 100
- 2007: 150
- 2008: 100

Recalls
Allergens of Concern

United States
– Peanuts
– Soy
– Milk
– Eggs
– Fish
– Shellfish
– Tree Nuts
– Wheat

Canada
– Peanuts
– Soy
– Milk
– Eggs
– Fish
– Shellfish
– Tree Nuts
– Wheat
– Sesame
– Sulfites
Food Ingredient Issues

- Oils
- Enzymes
- Gums
- Flavors/Extracts
- Lecithin
- Lactose
- Starch

- Gelatin
- Processing Aids
- Colorings
Why Monitor for Food Allergens?

4 types of Risks

1) Clinical
2) Regulatory
3) Consumer
4) Business
Types of Risk

• Clinical
  - Up to 8% of children, and up to 3% of adults in US have a food allergy
  - Researchers estimate 50,000 emergency room visits, and 150-200 deaths per year from food-induced anaphylaxis
  - Trace amounts (ppm) of the offending food will trigger reactions
What is Tolerance Level?

Current available data indicate that it is not possible to set a limit to the amount of allergenic protein there must be in a food to elicit an allergic reaction.
Types of Risk

• Regulatory
  - FALCPA:
    - declaration of allergens on label
    - ingredients, except those exempt, but including incidental additives, flavors, colors, mixes and processing aids.
    - “enforcement discretion” on soy lecithin
    - define gluten-free as < 20 PPM “prohibited grains” (wheat, rye, barley)
Types of Risk

• Consumer/Sales
  - dissatisfaction and overall loss of confidence
  - brand loyalty can be the enemy of the consumer
  - Proliferation of “May Contain…” type labels causing frustration and confusion to consumers
Considerations When Using Precautionary Labeling

- Is the presence of allergen documented through visual inspection or analytical testing?
- Is the risk of presence of unavoidable?
- Is the allergen is present in some, but not all product?
- Is the presence of allergen is potentially hazardous?
Types of Risk

• Business
  - increased costs due to down-time, change in product formulation and equipment change-over
  - loss of competitive edge in marketplace
  - Regulatory agencies will almost always investigate a facility that has had a recall
“Hidden Risks”

- Recycled CIP and COP rinses
- Oils re-contaminated after refining
- Rework
- Labeling terms i.e. non-dairy creamer
- Many others…
Elements of an Allergen Control Plan

- Allergen Risk Assessment
- Engineering and System Design
- Scheduling
- Processing Controls
- Maintenance
- Sanitation/Change Over Cleaning
- Labeling/Packaging
- Consumer Complaint Systems
- Training
- Auditing/Verification
Suppliers

• Ingredient suppliers should be evaluated and audited routinely for **Allergen Control Plans** and **Allergen Labeling Controls**

• *Certificates of Analysis* are provided, kept current and reviewed, and contain full disclosure of all ingredients used

• Prior notification of any formulation changes are provided by suppliers and kept on file

• Confirmation that unlabelled allergens are not in formulated multi-component ingredients i.e. spice blends
Ingredients/Raw Materials

Storage

• Incoming ingredients are clearly labeled with allergens
• Ingredients are given a specific lot # and tracked through system
• Designated storage areas for allergenic ingredients where feasible
  • If designated receiving area is not feasible, store allergenic ingredients below non-allergenic ingredients
• Use a color-coding system for totes and bins, and stickers for pallets and bulk ingredients
Equipment/Engineering

- Dead legs or dead spots can be areas that hold previous production.
- Review equipment annually, with documentation, for dead spots, rough surfaces and voids.
- Equipment should allow for thorough cleaning and be accessible for inspection.
- Avoid a situation where product flow may allow for cross-contamination, i.e., valve clusters, layered conveyors.
- Evaluate air handling system to insure it’s not a source for contamination.
Operations/Processing

Scheduling

• Introduce allergenic components into the process as late as possible
• Whenever possible, avoid manufacturing an allergenic product prior to a non-allergenic product
• Develop an Allergen Matrix or changeover grid to identify necessary sanitation and scheduling practices
• If using re-work, make sure to follow Exact-into-Exact philosophy
• Where possible, schedule long runs of allergenic product, and allow for full allergen clean-up when complete
Operations/Processing

Practice

• Do not allow the use of single-service items such as tray-liners to be reused
• When sampling the in-process product, be certain the sampling device is cleaned & sanitized appropriately between products
  • Double-check formula during scale-up
• Physical barriers such as shield covers and catch pans should be positioned to prevent spillage and cross-contamination
Labeling/Packaging

• Should have a label SOP to insure all necessary steps are taken for accuracy
• All formula changes are properly documented and synchronized between labeling and manufacturing
• All minor ingredients and processing aids that contain allergenic protein are properly reflected on the label
• Precautionary labeling, if used, is justified, not overused, and not a replacement for proper GMP's
Sanitation

Process

- Identify all equipment, conveyors and food contact surfaces that require cleaning after an allergen run
- Be sure to include splash zones, indirect product contact surfaces, utensils, employees and sampling equipment in the SSOP
- On pre-op inspections, make sure you specify allergen check points
- Focus on hard-to-clean areas, including valves, dead spots, pumps corners, welds and angles
- Consider using color-coded cleaning utensils
FDA “Guidance on Inspections…”

www.fda.gov/ICECI/Inspections/InspectionGuides

- Product Development
- Receiving
- Equipment
- Processing
- Final Product Testing
- Labeling
Neogen and Food Allergens

• Neogen joined FARRP in 1997 and collaborated on the industries first test kit for peanut allergen
• FARRP – Food Allergy Research and Resource Program, University of Nebraska
• FARRP is globally recognized in the food industry as a trusted advisor on allergen issues
• Later collaborations on development of rapid test kits for peanut, milk, egg, almond, hazelnut, gluten and soya
Designing a Suitable Antibody

Fermented and Hydrolyzed Proteins: Known Limitations
Developing the Right Antibody

Monoclonal or Polyclonal

Cooked or Raw

Rabbits, Sheep, Goats, Mice
Quantitation and Screening
Reveal 3-D Products
Allergen Specific Tests

- Almond
- Casein
- Egg
- Gluten
- Hazelnut
- Peanut
- Shellfish
- Soya

Reveal 3-D
Reveal 3-D™ Components

Components provided
(from top left to bottom right):
• Extraction buffer sachet
• Sample tube & cap
• RAPID 3-D test device
• swab
<table>
<thead>
<tr>
<th>Kit Format</th>
<th>Lateral Flow Device, 3-D Technology, no equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Detection</td>
<td>Low ppm levels of food residue</td>
</tr>
<tr>
<td>Suitable for Testing</td>
<td>environmental swabs and rinse solutions</td>
</tr>
<tr>
<td>Samples per kit</td>
<td>10 samples or swabs</td>
</tr>
<tr>
<td>Total test time</td>
<td>&lt;10 min per sample including extraction</td>
</tr>
</tbody>
</table>
Reveal 3-D Procedure

- Ensure samples are as homogenous as possible
- Avoid cross contamination during sample preparation
- Ensure assay steps are followed as outlined in insert
- Flows across line of more antibody-coated colloidal gold
- Antibody captures target
Reveal 3-D™ Test - Positive Result

Sample

Result Window

T Test
O Overload
C Control
Reveal 3-D™ Test - Negative Result

Sample

Result Window

T
Test

O
Overload

C
Control
We Stand Behind Our Results

**Reveal 3-D™ Test - Overload Result**

Sample

Result Window

T  Test

O  Overload

C  Control

© 1996 Nike Clark

NEOGEN CORPORATION
Reveal 3-D Test Results
for clear decision making

From Left to Right:
Negative; Positive; High Positive

HIGH POSITIVE
No line at O, faint or line absent at T (Overload)

POSITIVE
Any intensity of line at T - Above detection limit

NEGATIVE
No line at T - Undetectable
Veratox for Quantitation
Sample Preparation

Sample (5 grams) + 125 ml PBS (60 °C)

Shake in water bath for 15 min at 60 °C

Filter/Settle/Centrifuge

Run Test Kit (30 min)
Extract samples prior to running assay. Add controls and samples to appropriate wells.

Transfer to antibody wells and incubate 10 minutes.

Wash wells with wash buffer solution.
Add conjugate to all wells and incubate 10 minutes.

Wash wells with wash buffer solution.

Add substrate and incubate 10 minutes.
Add Red Stop.

Read results in a microwell reader.

Veratox Food Allergens
Sandwich ELISA Theory

Capture Antibody

Allergens

Detector Antibody

Substrate

Wash

Conjugate 10 min

Substrate 10 min

Stopping Reagent

Read (OD_{650\,nm})
Validation

• Once prototype is developed it must be validated
  • Cross Reactivity
  • Limit of Detection
  • Inter and Intra Assay Variability
  • Recovery
  • Stability
  • Food Trials (various processed conditions)
  • Alpha and Beta Testing
The Role of Rapid Test Kits

• Become “Standard of Care” in food industry
• A valuable tool, but know what to do with results.
• Understand power of final results and have a corrective action plan in place.
• Most not checking final product.
Limitations of Test Kits

All antibody-based tests

- Hydrolyzed proteins
  - Example: HVP, hydrolyzed egg protein
- Fermentation substrates
  - Examples: guar gums, xanthan gums, starter cultures, soy sauce
- Processing aids
  - Examples: lecithin, enzymes

Proteins from these products are generally not detectable on the test kits. However, allergenicity can remain.
## Know What You’re Detecting

<table>
<thead>
<tr>
<th>Neogen Test Kit</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>Total peanut protein</td>
</tr>
<tr>
<td>Egg</td>
<td>Raw and cooked egg whites</td>
</tr>
<tr>
<td>Milk</td>
<td>Casein and whey proteins</td>
</tr>
<tr>
<td>Almond</td>
<td>Total almond protein</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>Total hazelnut protein</td>
</tr>
<tr>
<td>Gliadin</td>
<td>Prolamins (gliadin, secalin, hordein)</td>
</tr>
<tr>
<td>Processed Soy</td>
<td>Most soy ingredients</td>
</tr>
</tbody>
</table>
Industry Usage of Test Kits

High Frequency
- Manufacturing issue diagnosis
- Sanitation validation

Medium Frequency
- Allergenic ingredient determination
- Consumer complaint sample

Low Frequency
- Routine finished product testing
- Supplier ingredient verification
Tools to simplify your HACCP

- Validation
- Verification
- Corrective actions
- Training
- Trouble-shooting
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